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## Quantitation of five organophosphorus nerve agent metabolites in serum using hydrophilic interaction liquid chromatography and tandem mass spectrometry

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### Abstract

Although nerve agent use is prohibited, concerns remain for human exposure to nerve agents during decommissioning, research, and warfare. Exposure can be detected through the analysis of the hydrolysis products in urine as well as blood. An analytical method to detect exposure to five nerve agents, including VX, VR (Russian VX), GB (sarin), GD (soman) and GF (cyclosarin), through the analysis of the hydrolysis products, which are the primary metabolites, in serum has been developed and characterized. This method uses solid phase extraction coupled with high performance liquid chromatography for separation and isotopic dilution tandem mass spectrometry for detection. An uncommon buffer of ammonium fluoride was used to enhance ionization and improve sensitivity when coupled with hydrophilic interaction liquid chromatography resulting in detection limits from 0.3–0.5 ng/mL. The assessment of two quality control samples demonstrated high accuracy (101–105%) and high precision (5–8%) for the detection of these five nerve agent hydrolysis products in serum.

### Keywords

Organophosphorus nerve agents; metabolites; serum; exposure

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## Introduction

Organophosphorus nerve agents are highly toxic compounds that were originally developed as potent pesticides in the 1930s. Since that initial discovery more nerve agents have been synthesized and stockpiled for warfare purposes. Although use of these compounds has been limited in recent years [1], concerns remain that nerve agents will be used for non-sanctioned warfare or terrorist activities. Stockpiles throughout the world are gradually being decommissioned [1] and laboratory research to improve treatments for exposed persons is being pursued [2, 3]. These activities may result in accidental human contact with nerve agents; therefore, the improved and expanded ability to assess human exposure to nerve agents is needed.

Following entry into the human body, nerve agents are either hydrolyzed or bound to acetylcholinesterase, butyrylcholinesterase, and other non-specific proteins. The binding to the acetylcholinesterase is the action that results in symptoms including miosis, seizures, and potentially death [1]. The hydrolysis products, also known as nerve agent metabolites, are readily excreted in the urine, but have also been detected in blood [4–6]. More specifically, human exposure to VX was identified in serum using gas chromatography/mass spectrometry (GC/MS) to measure the hydrolysis product, ethyl methylphosphonic acid. In this exposure case which resulted in death, the amount of metabolite from VX was determined to be 125 µg/mL [5]. Additionally, the hydrolysis products were detected in minipig blood using liquid chromatography-tandem mass spectrometry (LC/MS/MS) [4] following exposures to sarin and cyclosarin.

These hydrolysis compounds have been extensively quantitated in urine through various analytical methods. Gas chromatography coupled with mass spectrometry or tandem mass spectrometry has been used with success, although a derivatization step is required [7–9]. To avoid the additional derivatization, liquid chromatography coupled with mass spectrometry or tandem mass spectrometry has been applied, achieving sensitivities on the order of pg/mL in urine [10–13].

In most cases, sample preparation, including liquid-liquid extraction (LLE) and solid phase extraction (SPE), was necessary to achieve these low levels of detection. Solid phase extraction was cited more often than LLE, most likely due to the ease of use and potential for automation. The different solid phase extraction methods used for these compounds, included aqueous normal phase [10, 11], reversed phase [4], anion exchange [12, 14], and zirconia [14, 15]. However, for the extraction of nerve agent metabolites from serum, only LLE and reversed phase SPE were used [4–6].

An analysis method which includes the other nerve agent metabolites resulting from soman and VR exposure is needed, since only three nerve agent metabolites, EMPA, IMPA and CMPA, were previously quantitated in serum [4–6]. Although the amount of metabolite present in biological matrices has not been related to dose in humans, a quantitative value is still desired to obtain as much information regarding the relative amount of exposure as possible to differentiate between exposed and unexposed persons. Given this focus, an assay with high sensitivity is desired above a quantitative method designed to capture all exposure

levels. Additionally, this method should detect exposure to all five nerve agents within a single, high-throughput, and automated analysis. This study documents the development of a SPE-LC/MS/MS analysis for five nerve agent hydrolysis products in human serum which are indicative of exposure to sarin (GB), soman (GD), cyclosarin (GF), VX and VR.

## Methods and Materials

### Materials

The analytes evaluated for this method were the following: EMPA (ethyl methylphosphonic acid, CAS 1832-53-7), the metabolite of VX (*O*-ethyl *S*-(2-diisopropylaminoethyl) methylphosphonothioate, CAS 50782-69-9); IMPA (isopropyl methylphosphonic acid, CAS 1832-54-8), the metabolite of GB (isopropyl methylphosphonofluoridate CAS 107-44-8); PMPA (pinacolyl methylphosphonic acid, CAS 616-52-4), the metabolite of GD (pinacolyl methylphosphonofluoridate, CAS 96-64-0); CMPA (cyclohexyl methylphosphonic acid, CAS 1932-60-1), the metabolite of GF (cyclohexyl methylphosphonofluoridate, CAS 329-99-7); MMPA (2-(methyl)propyl methylphosphonic acid, CAS 1604-38-2), the metabolite of VR (*O*-2-(methyl)propyl *S*-2-(diethylaminoethyl) methylphosphonothioate, CAS 159939-87-4).

The internal standards were isotopically labeled as follows: EMPA, ethyl-D<sub>5</sub>; IMPA, isopropyl-<sup>13</sup>C<sub>3</sub>; PMPA, trimethylpropyl-<sup>13</sup>C<sub>6</sub>; CMPA, cyclohexyl-<sup>13</sup>C<sub>6</sub>; IMPA, methylphosphonyl-<sup>13</sup>C. The structures of the organophosphorus metabolites and internal standards are shown in Figure 1.

The analytical calibrators were prepared at concentrations of 0.5, 1.0, 2.0, 5.0, 10, 25, 50, 100 ng/mL of each analyte in serum; the quality control samples were prepared at 4.0 and 40 ng/mL of each analyte in serum. A primary stock (20 µg/mL of each analyte in methanol) purchased from Cerilliant Corporation (Round Rock, TX) was used to make stock solution I (4.0 µg/mL) and stock solution II (0.2 µg/mL). Stock solution I was prepared by aliquoting 400 µL 20 µg/mL stock to a glass vial containing 1600 µL of serum. The second stock solution was prepared by pipetting 100 µL of stock solution I to a glass vial containing 1900 µL of serum. Calibrators 0.5, 1.0, 2.0, and 5.0 ng/mL were made by aliquoting 25, 50, 100, 250 µL, respectively, of stock solution II to 10.0 mL volumetric flasks. Calibrators 10, 25, 50, and 100 ng/mL were prepared by delivering 25, 63, 125, and 250 µL, respectively, of stock solution I to their appropriate 10.0 mL volumetric flask. Quality control low (4.0 ng/mL) and quality control high (40 ng/mL) were prepared by adding 200 µL of stock solution II and 100 µL of stock solution I, respectively, to 10.0 mL volumetric flasks. An additional solution was prepared at 0.25 ng/mL in serum to evaluate the limits of detection for the assay, by delivering 17 µL of stock solution II to a 10.0 mL volumetric flask. Spiked samples, 0.75 and 3.0 ng/mL, were prepared by aliquoting 37.5, and 150 µL of stock solution II to their respective 10.0 mL volumetric flask. Spiked samples, 15, 70, and 90 ng/mL were prepared by delivering 37.5, 175, and 225 µL of stock solution I into their respective 10.0 mL volumetric flasks. All volumetric flasks were filled to 10.0 mL using serum purchased from Tennessee Blood Services Corporation (Memphis, TN). The serum was pooled from five donors, shipped at 4°C, received two days later, and subsequently

stored at  $-20^{\circ}\text{C}$  until use. Once prepared, all fortified samples, calibrators, and quality control samples were maintained at  $-20^{\circ}\text{C}$ .

Internal standard was prepared by dilution of a 500 ng/mL aqueous mixture (Cerilliant Corporation, Round Rock, TX) to a concentration of 23.8 ng/mL in DI water.

Organic-free 18.2 M $\Omega$  Type I water from a purifier purchased from Aqua Solutions, Inc. (Jasper, GA) was used in these studies. Pelletized 98% ammonium fluoride was purchased from Alfa Aesar (Ward Hill, MA) and molecular biology grade DEPC-treated 5M ammonium acetate from CalBiochem (La Jolla, CA). HPLC-grade acetonitrile and methanol were purchased from Tedia (Fairfield, Ohio). All human serum was purchased from Tennessee Blood Services Corporation (Memphis, TN).

### Sample preparation

To prepare samples for extraction, 25  $\mu\text{L}$  of 23.8 ng/mL internal standard was aliquoted into each well of a 2 ml 96-well NUNC plate. Fifty microliters of well-mixed serum was added to the internal standard, followed by 1000  $\mu\text{L}$  of acetonitrile. The plate was sealed using a sheet of Thermo Scientific Adhesive PCR Sealing Foil (Hudson, NH) and vortexed for 5 minutes on a ThermoLab Systems Wellmix (Hudson, NH). The NUNC plate was then centrifuged for 5 minutes at 3000 rpm.

The solid phase extraction was automated by using the Life Science Zephyr (Hopkinton, MA). The Phenomenex Strata Si-1 SPE 96-well plate (55  $\mu\text{M}$ , 70  $\text{\AA}$ , 100 mg) was pretreated with 1000  $\mu\text{L}$  of 25% water in acetonitrile, followed by 1000  $\mu\text{L}$  of acetonitrile. The entire sample mixture with precipitate was then loaded to the SPE 96-well plate, and rinsed by a two-step process of 1000  $\mu\text{L}$  of acetonitrile, followed by 1000  $\mu\text{L}$  of 7% water in acetonitrile. The solutions were pulled through the SPE 96-well plate using vacuum. The analytes were then eluted with 1000  $\mu\text{L}$  of 28% water in acetonitrile and collected in a clean 96-well NUNC plate.

The eluent was heated at  $70^{\circ}\text{C}$  under 25 psi of nitrogen in a TurboVap for 30 minutes and then evaporated to dryness after increasing flow rate to 70 psi. The lower initial flow rate eliminated solvent splashing. The sample was then reconstituted in 100  $\mu\text{L}$  of 5% water in acetonitrile, mixed by pipetting twice, and transferred to a 150  $\mu\text{L}$  96-well PCR plate and sealed.

### Instrumental analysis

The HPLC separation was performed using an Agilent 1200 HPLC with a well-plate autosampler (Santa Clara, CA). A Waters Atlantis<sup>®</sup> HILIC 2.1-mm  $\times$  50-mm with 3- $\mu\text{m}$  particles (70% porosity) HPLC column was used (Milford, MA). This hydrophilic interaction chromatography (HILIC) column consists of high purity, non-bonded silica particles. The injector was programmed to draw 20  $\mu\text{L}$  of sample and wash the injector needle for 10 s in the wash port using 50% water in acetonitrile. The sample was then injected onto the column using an isocratic mobile phase consisting of 92% acetonitrile and 8% 1.0 mM ammonium fluoride with an initial flow rate of 500  $\mu\text{L}/\text{min}$ . Following elution of the analytes, the flow rate was increased to 1000  $\mu\text{L}/\text{min}$  at 3.01 min to remove any late eluting

impurities. The flow rate returned to 500  $\mu\text{L}/\text{min}$  at 5.01 min to ensure stable pressure for the following injection. This method allows a 5-min injection-to-injection cycle time.

The mass spectrometric analysis was performed using an API 4000 triple quadrupole QTrap mass spectrometer from Applied Biosystems (Foster City, CA) controlled by Analyst software. The mass spectrometer was operated in multiple-reaction-monitoring (MRM) mode using negative electrospray ionization with assisted heating. The specific operating conditions are listed in Table 1 with the proposed fragment ions.

The specific settings used were curtain gas (CUR), 10 psi; nebulizer gas (GS1), 40 psi; turbo gas (GS2), 40 psi; GS2 temperature (TEM), 550  $^{\circ}\text{C}$ ; collision gas, nitrogen; collision gas (CAD), Medium, producing a gas pressure reading of  $3.3 \times 10^{-5}$  Torr; ionspray potential (IS),  $-4500$  V; entrance potential (EP),  $-10$ ; interface heater (IHE), on.

The data were analyzed using Analyst 1.5.2, which was provided with the instrument. This software is used to review the chromatograms for retention times, baselines and possible interferences. Quantitative analysis of the data by automated and manual integrations, linear regression, and calculation of accuracies and correlation coefficients was also performed with this software package. The chromatographic data were smoothed three times prior to integration with a bunching factor between 1 and 3, and fitted by linear regression using  $1/x$  weighting.

### Safety precautions

The techniques and materials in this method do not pose any special hazards. General considerations include exercising universal precautions, such as wearing appropriate personal protective equipment, when handling chemicals and serum samples. The high voltage employed in electrospray ionization should also be considered a hazard, and the safety interlocks provided by the instrument manufacturer should not be altered. For instrument-specific safety concerns, please consult the manufacturer.

## Results and Discussion

### Sample Preparation

Initial evaluation of a protein precipitation using acetonitrile or acetone for the sample preparation of these compounds from serum resulted in lower sensitivities than desired. As exposures may be small and/or a sample may be collected many hours to days following exposure, it is important to have the highest sensitivity possible. Additionally, the presence of interfering matrix components was detected in select transitions, which would negatively impact the specificity of the analysis. To accomplish the goals of sensitivity and specificity, additional sample preparation was necessary.

Solid phase extraction was selected for sample preparation due to automation and high throughput potentials. The initial solvents and volumes used for the extraction were selected from Swaim, et al [10] from urine sample extraction. The sorbent was conditioned with 1000  $\mu\text{L}$  of 25% water in acetonitrile, followed by 1000  $\mu\text{L}$  of acetonitrile. After the sample mixture, comprised of 100  $\mu\text{L}$  of serum, 25  $\mu\text{L}$  of internal standard solution and 1000  $\mu\text{L}$  of

acetonitrile) was loaded, the sorbent was rinsed with 1000  $\mu$ L of acetonitrile, followed by 1000  $\mu$ L of 10% water in acetonitrile. Finally, the compounds were eluted using 1000  $\mu$ L of 25% water in acetonitrile and collected in a 2 mL 96-well NUNC plate. Using a serum matrix fortified at 10 ng/mL, each step within the solid phase extraction protocol was then evaluated in triplicate for optimal recovery. To evaluate the loading step, 50  $\mu$ L of serum was diluted with acetonitrile, with additional deionized water as needed, to yield an aqueous solution of 7, 12 and 17%. The seven percent aqueous loading solution resulted in the highest responses with no detected breakthrough. Next, the second wash step was assessed at acetonitrile content ranging from 93 – 83%. The wash step of 93% acetonitrile yielded the highest recoveries with minimal losses detected. No adjustments were made to the elution composition as complete elution was achieved in one step with the 25% water/75% acetonitrile mixture. Final extraction recoveries, determined at 10.0 ng/mL and calculated by the ratio of the measured concentration to the spiked concentration, were as follows: EMPA, 88% (standard deviation of 17), IMPA, 76%(13) MMPA , 92% (9.6)), PMPA, 94% (10), and CMPA , 95% (7.7). With the optimized solid phase extraction parameters, no analyte was detected within the two captured wash steps. Following the elution, a second elution step was evaluated to remove the analytes still remaining on the SPE. This second elution showed minimal (<1%) amounts of PMPA, CMPA and MMPA, approximately 5% of EMPA and no detectable IMPA remained on the SPE following the initial elution.

### Separation and Detection

Previous studies have reported successful separation of these compounds with HILIC chromatography using an isocratic ammonium acetate buffer coupled with acetonitrile (14% 20 mM ammonium acetate: 86% acetonitrile) as the mobile phase [10, 11]. This mobile phase composition was used as a starting point for the separation of these compounds and is shown in Figure 2b. Since the mass spectrometric intensity of these compounds has been augmented with the addition of various solvents [16] post column, alternative buffers were investigated to determine if increases in response could be achieved. Ammonium fluoride has been reported to result in the ionization of additional compounds and may in fact enhance ionization when using negative electrospray [17, 18]. With this information the HILIC chromatographic separation was evaluated replacing the 20 mM ammonium acetate buffer with 1 mM ammonium fluoride buffer. This change resulted in an increased signal for all compounds (Table 2), with an average 7.7-fold improvement in peak area response, with a standard deviation of the peak area response between the compounds was found to be 0.77. Using the 1 mM ammonium fluoride increased retention; therefore, to minimize runtime, the isocratic mobile phase composition was adjusted to 8% 1 mM ammonium fluoride and 92% acetonitrile to achieve retention factors of 4.6–6.1 (Figure 2a). This change also resulted in further 2-fold signal increase from the 14% ammonium fluoride mobile phase, which is equivalent to at least a 10-fold signal increase (Table 2), indicating the impact of higher acetonitrile content on the ionization efficiency of these compounds.

### Method Characterization

Twenty sets of calibrators with QC samples were extracted and analyzed by LC-MS/MS to determine the precision and bias of this method over time. Multiple analysts prepared no more than two standard sets with QC samples per day over a period of 36 days. Quality



control characterization data are presented in Table 3. The inter-day relative standard deviations for both quality control samples for all five compounds were below 8% and the accuracy was between 101–105% for all compounds. These results confirm this method provides acceptable precision and accuracy for these compounds according to the FDA Bioanalytical Method Development Guidelines [19].

### Limits of Detection

The limits of detection (LOD) for the five compounds were estimated using low level spiked serum with concentrations from 0.25 – 1.0 ng/mL. Each low level spiked sample was analyzed 20 times within a two-week period, with no more than five replicates within one day. The absolute values of the standard deviations were then plotted versus concentration with the intercept of the least-squares fit of this line equal to  $S_0$  with  $3S_0$  being the LOD [20]. The LODs for this method were determined and reported in Table 3. These estimates, ranging from 0.25–0.50 ng/mL, exceed other methods developed for serum which reported limits of detection from 3.9 – 30 ng/mL for EMPA[5, 6] and 16 ng/mL for PMPA and CMPA [7].

### Sample Analysis

One hundred individual serum samples were extracted and analyzed to evaluate the presence of nerve agent metabolites in the serum of persons with no known exposure. A representative sample is shown in Figure 3. No peaks were observed above the lowest calibration point; therefore, results above the detection limit would indicate that an exposure to these compounds has occurred

To evaluate accuracy and precision across the calibration range, serum samples were prepared at the following concentrations: 0.75, 3, 15, 70, and 90 ng/mL. These samples were extracted and analyzed five times to generate the statistical data presented in Table 4. The relative standard deviations for all five levels were less than 10%, with the exception of the 0.75 ng/mL spike of IMPA which resulted in a variance of 18%. Similarly, the four highest levels resulted in accuracy between 96% and 112%. However, for the lowest spiked sample at a concentration of 0.75 ng/mL the accuracies were between 78–93%. This drop in accuracy and increase in variability at the low concentration may be due to the closeness of the value to the limit of detection.

To widen application of this method to samples with a metabolite concentration greater than 100 ng/mL, a 4.0 µg/mL fortified sample was prepared in serum. This sample was diluted to 4.0 and 40.0 ng/mL using blank serum; the diluted samples were then extracted and analyzed in triplicate. The average quantitated values ranged from 3.91–4.27 ng/mL and 39.5–41.8 ng/mL for the two levels. When the quantitated values were calculated with the dilution factor, the accuracies ranged from 97.8–106%.

The stability of these compounds in serum stored for 6 months at –20°C was evaluated at 4.0 ng/mL and 40.0 ng/mL. The stored samples were analyzed in triplicate and quantitated relative to freshly prepared calibrators. The concentrations of the two levels were within the 10.0% of the original spiked concentration for all five analytes. Given the variability of the

method, these results indicated that the metabolites were stable for at least 6 months stored frozen.

### Limitations

Due to a high background response resulting from the ammonium fluoride buffer, the initial transition selected for CMPA (177>79 m/z) did not result in a discernible peak within the reportable range. An alternative transition of 177>63 m/z was evaluated; however, this new transition was only detectable to about 1 ng/mL due to limited signal. Similarly, the confirmatory ion for PMPA was not consistently identified below 2 ng/mL due to a high background response. Previous publications have indicated that this background may be attributed to the impurities within the ammonium fluoride used for the buffer and potentially the interaction of this buffer with the stationary phase [18]. It should be noted that these issues were only detected in the confirmatory ions and do not affect the reported limits of detection for these compounds

### Conclusions

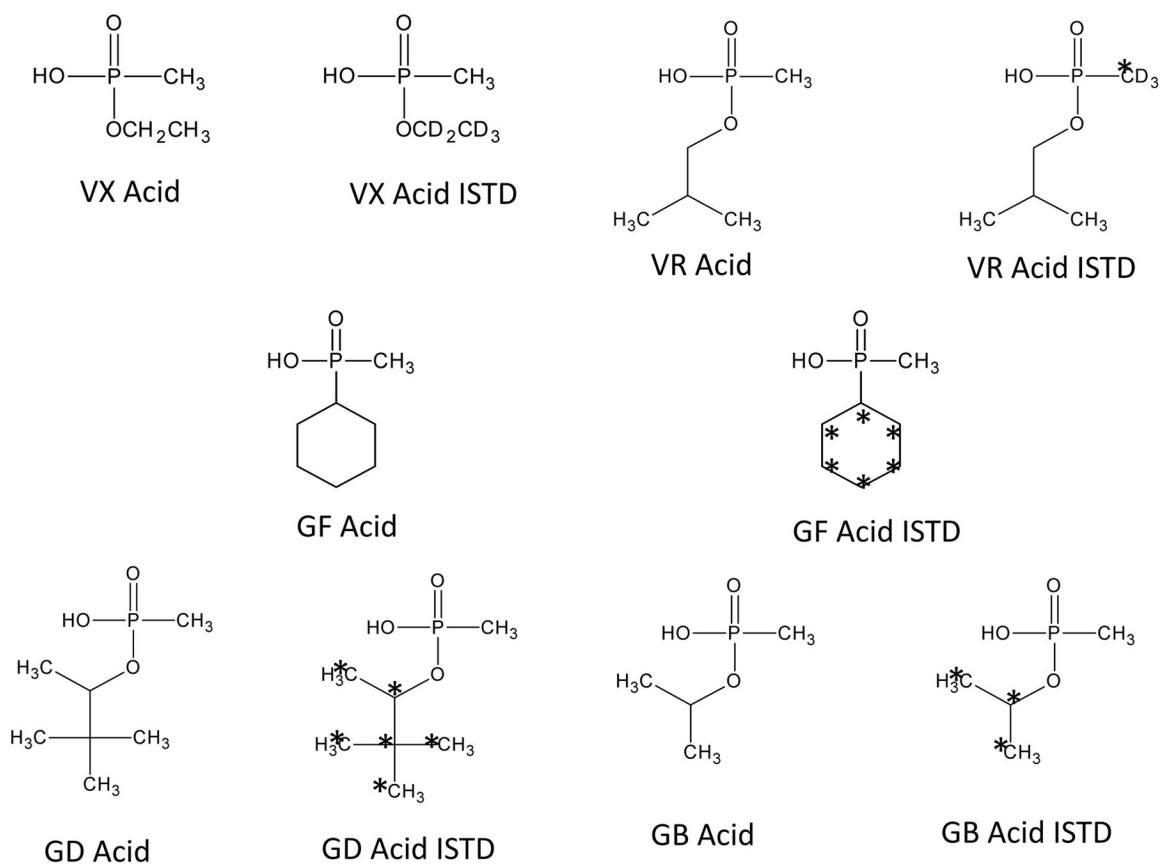
A method to detect exposure to five nerve agents, including VX, VR, soman (GD), sarin (GB), and cyclosarin (GF) in serum has been developed and evaluated. The novel use of ammonium fluoride as the mobile phase buffer increased the ionization of the compounds up to 10-fold over the use of ammonium acetate, which substantially increased the sensitivity of this assay. Both the resulting precision and accuracy, 8% and 100–105% respectively, were within FDA recommendations. Since no peaks were detected within 100 individual serum samples with no known exposure to nerve agents, a response above the limit of detection is indicative of exposure to nerve agents. Given the potential for blood to clot and lyse, often due to poor handling or storage, which would impact the quality of the serum, future studies are planned to apply this assay to quantitate nerve agent exposure in non-ideal blood matrices.

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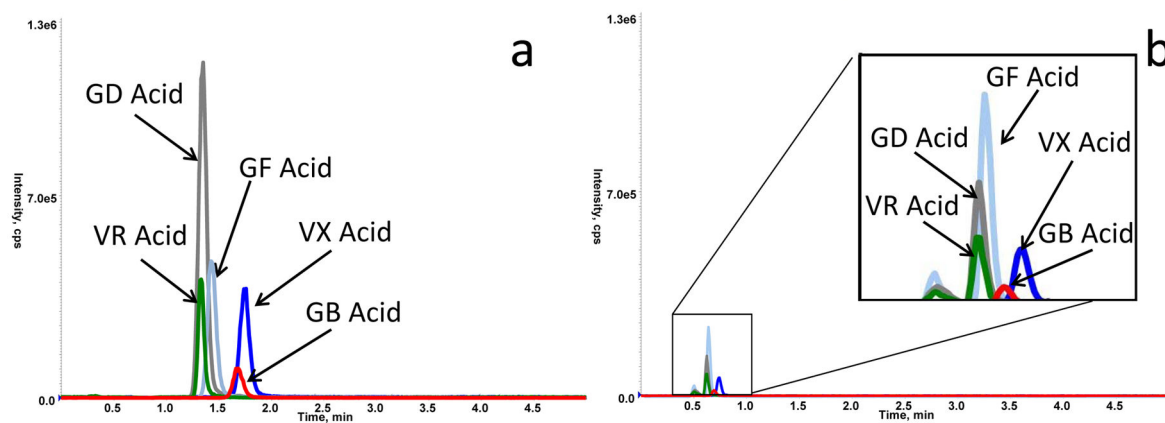
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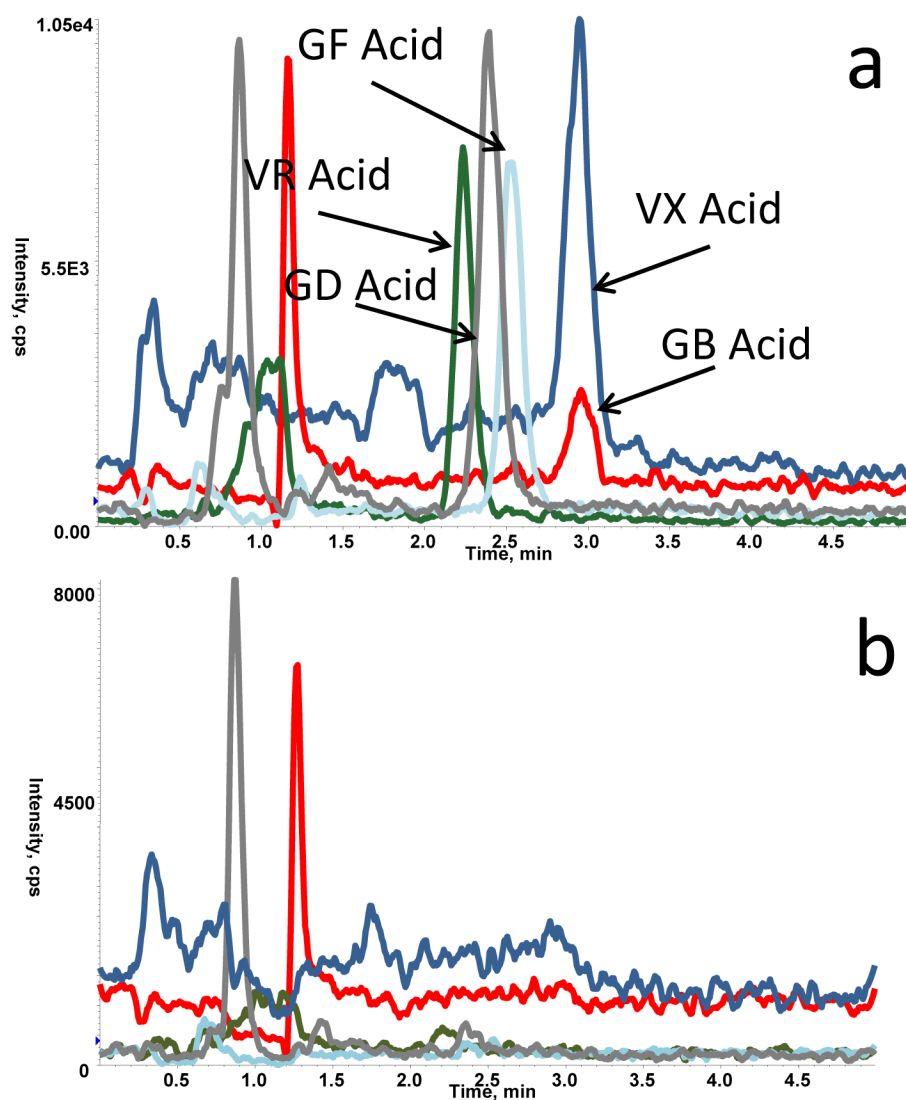


**Figure 1.**  
Structures of nerve agent metabolites and corresponding internal standards, \* = <sup>13</sup>C



**Figure 2.**

Chromatograms of 100 ng/mL calibrator in serum containing five nerve agent metabolites in different mobile phase compositions: a) 8% ammonium fluoride (1 mM)/92% acetonitrile, and b) 14% ammonium acetate (20 mM)/86% acetonitrile



**Figure 3.** Chromatograms of 0.5 ng/mL serum calibrator containing five nerve agent metabolites (a) and blank serum (b)

**Table 1**

Mass spectrometric transitions and parameters

Analyte	Precursor Ion (m/z)	Product Ion (m/z)	Declustering Potential (V)*	Collision Energy (V)*	Collision Cell Exit Potential (V)*
EMPA-Quantitation	123	95	-50	-18	-7
EMPA-Confirmation	123	79	-50	-36	-11
EMPA-Internal std	128	96	-50	-20	-7
IMPA-Quantitation	137	95	-55	-20	-7
IMPA-Confirmation	137	79	-55	-54	-11
IMPA-Internal std	140	95	-55	-20	-7
MMPA-Quantitation	151	95	-70	-22	-13
MMPA-Confirmation	151	77	-70	-28	-13
MMPA-Internal std	155	99	-70	-22	-13
PMPA-Quantitation	179.1	95	-50	-24	-15
PMPA-Confirmation	179.1	77	-50	-46	-13
PMPA-Internal std	185.1	95	-50	-24	-15
CMPA-Quantitation	177.1	95	-60	-28	-15
CMPA-Confirmation	177.1	63	-60	-54	-15
CMPA-Internal std	183.1	95	-60	-28	-15

**Table 2**

Percent of peak area response relative to the buffer composition of 14% ammonium acetate (20 mM) with 86% acetonitrile

	Ammonium fluoride (1 mM, 14%) with 86% acetonitrile	Ammonium fluoride (1 mM, 8%) with 92% acetonitrile
EMPA	7.46%	13.5%
IMPA	7.24%	13.4%
MMPA	8.42%	12.3%
PMPA	8.68%	12.1%
CPMA	6.92%	10.1%



**Table 3**

Mean, precision (relative standard deviation), accuracy, and limit of detection (LOD) values for five nerve agent metabolites in serum (n=20)

Analyte	Sample	Mean	Precision	Accuracy	LOD
IMPA	QC Low	4.07	8.24%	101.7%	0.4
	QC High	40.2	5.51%	100.4%	
PMPA	QC Low	4.20	5.53%	104.9%	0.3
	QC High	40.85	6.37%	101.9%	
CMPA	QC Low	4.13	5.41%	103.3%	0.5
	QC High	40.7	6.10%	101.6%	
EMPA	QC Low	4.07	7.03%	101.6%	0.5
	QC High	40.3	6.21%	101.0%	
MMPA	QC Low	4.06	5.18%	101.4%	0.4
	QC High	40.5	5.72%	101.2%	

**Table 4**

Mean, precision, and accuracy of spiked serum samples (n=5)

		VX Acid	GB Acid	VR Acid	GD Acid	GF Acid
0.75 ng/mL	Mean	0.58	0.61	0.64	0.66	0.70
	Precision	5.3%	18.1%	9.9%	6.0%	2.4%
	Accuracy	77.7%	81.8%	85.5%	88.0%	93.1%
3 ng/mL	Mean	3.22	2.87	3.04	3.16	3.23
	Precision	3.0%	4.3%	3.9%	3.5%	3.1%
	Accuracy	107.3%	95.8%	101.3%	105.5%	107.7%
15 ng/mL	Mean	16.6	16.2	15.8	16.7	16.7
	Precision	8.2%	8.0%	8.1%	8.9%	8.0%
	Accuracy	110.8%	108.0%	105.1%	111.6%	111.1%
70 ng/mL	Mean	78.1	76.1	74.7	77.6	77.5
	Precision	4.4%	4.6%	5.0%	4.5%	4.3%
	Accuracy	111.5%	108.7%	106.8%	110.8%	110.8%
90 ng/mL	Mean	99.8	96.7	95.6	98.2	99.7
	Precision	1.9%	2.8%	2.6%	3.2%	3.7%
	Accuracy	110.9%	107.4%	106.2%	109.1%	110.8%